Expression of a Recombinant Zika Virus E Domain III Protein by *E. coli* System

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INTRODUCTION

Zika virus (ZIKV) is a positive-sense, single-stranded RNA arbovirus belonging to the genus *Flavivirus* in the family *Flaviviridae*. ZIKV transmission primarily occurs by the bite of an infected *Aedes* mosquito (*Ae. aegypti* and *Ae. albopictus*) but can also occur by sexual intercourse, pregnancy (mother-to-child), and blood transfusions. Most individuals infected with ZIKV are asymptomatic, but 20-25% of those infected develop a mild and self-limited illness. Symptoms include fever, arthralgia, myalgia, conjunctivitis, and skin rash. Severe cases of ZIKV have occurred in Micronesia, French Polynesia, and South America where ZIKV has been linked to Guillain-Barré syndrome and microcephaly. Guillain-Barré syndrome is an autoimmune disorder that causes muscle weakness in legs and arms, paralysis, and, in severe cases, death. Microcephaly is a birth defect describing a smaller-than-normal head size for infants. ZIKV and dengue virus (DENV) have similar genomes encoding for proteins that make up the virus. The immune system produces antibodies against these proteins and can be cross-reactive between ZIKV and DENV. Protein identity for envelope (E) domain III is different between ZIKV and DENV by protein alignment. Antibodies against ZIKV E domain III are specific to ZIKV and have high neutralizing levels.

OBJECTIVE

The objective of this study was to express a recombinant ZIKV E domain III protein using an E. coli system. Because antibodies against ZIKV E domain III protein are highly specific to ZIKV, the protein may be useful for differential diagnosis between ZIKV and DENV and could be used for development of a rapid test kit.

MATERIALS AND METHODS

1. Creation of *E. coli* starter

One colony BL21 (DE3) pLysS E. coli was inoculated in LB broth with antibiotics *(E. coli* starter). The *E. coli* starter was incubated overnight.

2. Extraction of plasmid from *E. coli* starter and PCR

The recombinant plasmid was extracted from the *E. coli* starter. The extracted plasmid was amplified by PCR. After amplification, the PCR product was analyzed by gel electrophoresis and ethidium bromide staining. The positive band containing the ZIKV domain III gene was expected around 370-bp.

3. Expression of recombinant protein

The *E. coli* starter with the recombinant plasmid was added to LB broth-antibiotic (*E. coli* culture). The *E. coli* culture was incubated until OD at 600 nm reached 0.2-0.4, and some *E. coli* culture was collected (pre-induction). The remaining *E. coli* culture was induced by IPTG and incubated for 3 hours. After incubation, some *E. coli* culture was collected (post-induction). Pre- and post-induction cultures were centrifuged to collect the *E. coli* pellet. The pellet was re-suspended with 1X PBS and sonicated on ice. The cell lysate was centrifuged. Four fractions were obtained: pre-induction supernatant, pre-induction pellet, post-induction supernatant (soluble protein), and post-induction pellet (insoluble protein).

4. Analysis of protein expression

The recombinant protein was separated by SDS-PAGE gel electrophoresis. After electrophoresis, the gel was stained with Coomassie blue to determine the presence of proteins in sample fractions. Another SDS-PAGE gel with the same sample fractions was blotted onto a nitrocellulose membrane to detect the recombinant protein. Anti-histidine was used to detect the six-histidine motif attached to the recombinant protein. The expected size of the recombinant protein is around 16kDa.

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Figure 1. Infant with Microcephaly (Culjat M et al, *Clin Infect Dis*, 2016, PMID: 27193747)

Based on the attempted expression of the recombinant ZIKV E domain III protein, developing ZIKV serodiagnostic assays can be challenging

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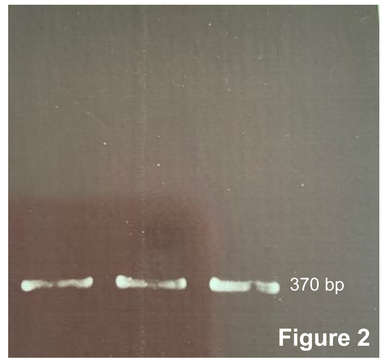
RESULTS

Figure 2. Ethidium bromide staining reveals *E. coli* starter contains recombinant plasmid.
Figure 2 displays the visualization of the agarose gel stained with ethidium bromide. The *E. coli* starter showed a positive band around 370-bp (ladder not displayed), which is the same size as the band for the positive control. This is also the expected size of the recombinant plasmid. There was no band for the negative control.

Legend:
Lane 1: *E. coli* starter Lane 3: Positive control
Lane 2: *E. coli* starter Lane 4: Negative control



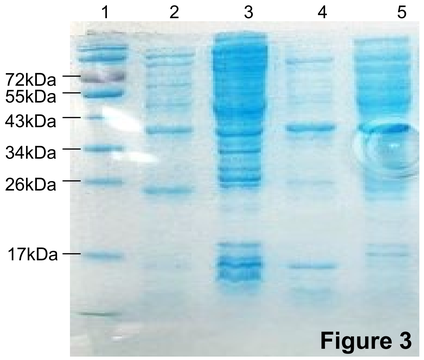


Figure 3. Protein is present in sample fractions.
Figure 3 displays the Coomassie blue staining of the SDS-PAGE gel. All sample fractions have protein.

Legend:
Lane 1: protein marker
Lane 2: pre-induction supernatant
Lane 3: pre-induction pellet
Lane 4: post-induction supernatant (soluble protein)
Lane 5: post-induction pellet (insoluble protein)

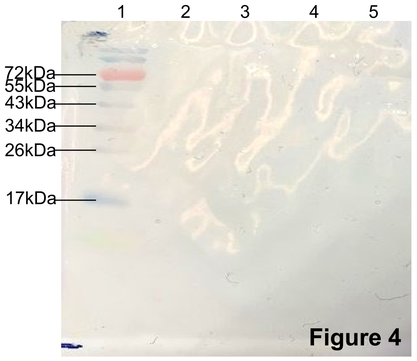


Figure 4. Recombinant ZIKV E domain III protein was not detected.
Figure 4 shows the results of the immunoblot. The membrane was reacted with anti-histidine to detect the six-histidine motif attached to the recombinant protein. The expected protein should be around 17-kDa. After reaction with anti-histidine, no recombinant protein was detected.

Legend: same as figure 2

CONCLUSIONS

The recombinant ZIKV E domain III protein was not expressed despite using *E. coli* transformed with the plasmid. This may be due to multiple issues. For example, IPTG may not have been functioning correctly, leading to no transcription of the ZIKV E domain III gene. The anti-histidine system may have also malfunctioned and not detected the recombinant protein. Instrument error may have also occurred with the blotting system. Unfortunately, there was not enough time to determine why the recombinant protein was not expressed.

Future directions include determining the issues with protein expression, using a different plasmid, or using a different gene. Based on the attempted expression of the recombinant ZIKV E domain III protein, developing ZIKV serodiagnostic assays can be challenging.